### NAVAL HEALTH RESEARCH CENTER

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#### Summary

#### Problem.

The use of dietary supplements and ergogenic aids within contemporary society is pandemic. One such supplement that appears to enjoy widespread use among SECWAR personnel and elite athletes is the compound creatine (CR). In some laboratory studies, this compound demonstrates ergogenic properties, particularly exercise protocols requiring repeated high intensity anaerobic exercise. Although laboratory studies have demonstrated an effect due to CR supplementation, the ability of CR supplementation to enhance or sustain work under operational conditions is less well established.

#### Objective.

The objective of this investigation was to determine if five days of CR supplementation could significantly improve the performance of a military task of active duty US Navy Sea Air and Land (SEALs) personnel.

#### Approach.

Twenty-four active duty SEALs were randomly assigned to a CR group (n=12) and a placebo (PL) group (n=12). The CR group supplemented their diets with 20 g/d of creatine monohydrate for 5 days. The PL group supplemented their diets with 20 g/d of a glucose polymer. The CR group had not taken any creatine or creatine-like substances for at least 30-days prior to the initiation of the study. Each of the study participants had their body composition and certain physiological variables measured before and after the supplemental trial period. The military task used as a criterion variable for performance consisted of the time taken to complete an abbreviated obstacle course (OC). Each participant was encouraged to complete the course in an all-out effort.

#### Results.

The time taken by all of the subjects was about 2 min requiring primarily anaerobic type exercise. Both the PL and the CR group improved their OC run times when compared to their corresponding control values (CR group = 4.5% and PL group = 3.2%). The blood lactate levels at 5-min and 10-min post completion of the OC run was similar for the control and the post-treatment time trials. Neither the OC run times nor any of the measured physiological variables was significantly different between the CR and PL groups after CR supplementation.

#### Conclusion.

Augmenting the diets of US Navy SEALs with 20 g/d of creatine monohydrate had no effect on the performance of a military task, body composition or physiological variables. The small differences in performance seen in some laboratory studies do not appear to carry over to the performance of a military task.

#### Introduction

The use of dietary supplements to augment health and performance has gained popularity among individuals within contemporary society (24). The use of dietary supplements and other ergogenic aids seems to be especially prevalent among athletes seeking to gain a competitive edge (13). The idea of a competitive edge is not only relevant to the athletic arena, but also has relevance in a military setting. The elite athlete and elite military special operations personnel devote a great deal of time to physical training and encounter immense physical demands during training. Both groups share a desire to gain an "edge" over opponents and are eager consumers of ergogenic aids and dietary supplements. The magnitude of dietary supplement use among U.S. Navy Sea Air and Land (SEAL) personnel is indicated by the results of a recent survey. Seventy-eight percent of 91 Navy special operations personnel surveyed indicated that they take some form of dietary supplement for the purposes of enhancing health and performance (38). There is little question among sports nutritionists that sound nutritional practices can optimize athletic performance and health, but whether this state of optimization can be reached through the use of dietary supplements remains an open question (10). Several published investigations and reviews have indicated that certain dietary practices, as well as specific nutrients, have the potential for enhancing exercise performance. (8,19).

One substance used as an ergogenic aid that appears to be used widely among athletes and military special operations personnel is creatine monohydrate. Creatine (Cr) is a normal chemical constituent of skeletal muscle (SKM) and exists in equilibrium as free Cr and in combination with phosphate as creatine phosphate (PCr). The sum of both forms of Cr is referred to as total creatine (TCr). The relative proportion of PCr:Cr in normal resting SKM is about 2:1 (4). In humans, Cr is synthesized endogenously by the liver, kidneys, and pancreas and carried to SKM where it is actively transported in-situ (37). Cr is also obtained through dietary sources (usually meat) where 1 to 2 g of Cr are consumed daily, an amount sufficient to replace the normal daily turnover (18). About 95% of the body's TCr is located in SKM; the remaining 5% is distributed among the heart, central nervous system, and testes (46).

During intense muscle contraction, PCr has been shown to rapidly decline. The decline in intramuscular PCr has also been associated with muscle fatigue and reduced force generation (25,40). Elevated intramuscular PCr, as well as Cr, may augment performance by increasing substrate availability for the enzyme creatine phosphokinase (CK) leading to a greater rate of ATP resynthesis. Since muscular levels of PCr have been associated with muscle contraction and fatigue, an elevated PCr level prior to exercise could be postulated to have the effect of enhancing exercise performance by reducing the rate of ATP depletion (29). The performance enhancing effects of increasing the rate of ATP synthesis would be most evident during intermittent or repeated intense physical activity by providing a more rapid replenishment of the endogenous ATP pool (12,21). The rapid replacement of ATP could also buffer against high levels of hydrogen ion concentrations, which has been associated with fatigue and declining force production (31). Another suggested explanation as to why supplemental Cr may enhance performance is that Cr has been hypothesized to stimulate synthesis of contractile proteins, particularly in type II muscle fibers (5,39).

Several published reports have established that dietary Cr supplementation of 20 to 30 g/d for 5 to 6 days will augment intramuscular Cr, PCr, PCr/ATP, and TCr (21,34). The greatest rate of accumulation appears to be during the first few days of supplementation where most individuals can acquire 20% to 30% more intramuscular Cr (21). There does appear to be an upper limit for the SKM's ability to concentrate Cr; a plateau is reached at about 160 mmol/kg dry mass. Once this maximum has been obtained, a supplemental Cr dose of 2 to 5 g/d has been shown to sustain elevated levels of Cr and PCr for 8 to 20 weeks (26,43,44). Based on these observations, dietary Cr supplementation appears to have many of the characteristics outlined by the Committee on Military Nutrition Research (CMNR) that qualify it as a true ergogenic substance (30).

Peak power performance indices and time to exhaustion have been shown to increase significantly with repeated or intermittent bouts of maximal cycling exercise (3,6,9), repeated bouts of jumping exercise, and treadmill running time to exhaustion (7). Other studies, however, have not shown any significant changes in exercise performance with

Cr supplementation during a single bout of the maximal cycling exercise (16,34) or maximal high-intensity treadmill running (15). Likewise, Cr supplementation had no effect on repeated 60 m sprint running velocities or run-times for repeated 700 m running bouts (36,42).

In most cases, enhanced performance is observed under well-controlled laboratory conditions. Those studies (usually swimming and running) conducted under more "field-like conditions" are less likely to demonstrate a significant effect due to Cr supplementation. The most consistent condition in which statistically significant increases in exercise performance can be demonstrated are those studies using repeated or intermittent, high-intensity anaerobic exercise of short duration. Cr supplementation does not appear to be of any particular benefit for the athlete performing aerobic endurance-type exercise (17,19,41).

Even though improved exercise performance has been demonstrated under certain laboratory conditions, the beneficial effects of Cr supplementation has not been established in the applied or operational setting. Therefore, we designed a study that would evaluate the efficacy of Cr supplementation to augment performance of a military task conducted under field conditions. The task used in this study was an abbreviated obstacle course (OC) run that is a standard component of U.S. Navy SEAL training. The abbreviated OC combined four elements that required repeated high-intensity explosive power conducted under mostly anaerobic conditions (approximately 2 min to complete).

#### Methods

**Subjects**. Twenty-four male SEALs participated in the study. The subjects ranged in age from 23 to 42 years. Each participant read and signed an informed consent form approved by the Naval Health Research Center's Committee for the Protection of Human Subjects. In addition, each subject was asked to complete a standard medical history questionnaire, an activity questionnaire, and provide a 10-lead ECG trace taken within the last 7 days. All subjects were in excellent health and had not taken any dietary

supplements containing Cr or Cr-like substances for a minimum of 30 days prior to entry into the study.

Subject Characteristics. Each study participant had their body weight determined using a standard electrical scale measuring to the nearest 0.1kg. Height was determined with a statometer and rounded to the nearest 0.1 cm. Peak aerobic capacity (VO<sub>2peak</sub>) was estimated by following a modified Balke protocol using a motorized treadmill and open-circuit spirometry to estimate whole-body oxygen uptake and carbon dioxide production (Sensormedics 2900 metabolic cart). Percent body fat was estimated from skinfold thicknesses taken at seven sites (27). Total body water, fat free mass, and percent body fat were also estimated by bioelectric impedance analysis (BIA; XITRON<sup>TM</sup>). Table 1 contains a summary of the physical characteristics of the subjects.

Table 1. Physical Characteristics of Subjects								
<u>Variable</u>	CR Group	<u>SEM</u>	PL Group	<u>SEM</u>	$\mathbf{P}(\mathbf{t})$			
Age (years)	*29.7	1.6	33.8	1.8	0.09			
Height (cm)	176	1.6	178.	1.3	ns			
Body Wt. (kg)	78.2	2.2	78.9	1.6	ns			
Body Fat (%) (BIA)	12.8	1.9	18.6	1.5	0.03			
Fat Free Mass (kg)	68.4	2.7	64.1	1.4	ns			
Peak VO <sub>2</sub> (ml/kg/min)	59.8	1.5	58.0	1.1	ns			
Peak Heart Rate (beats/min)	191	3.0	185	2.4	ns			

Abbreviations: CR = Creatine, PL = Placebo, SEM = Standard Error of the Mean, P(t) = two tailed independent t-test for equality of means, cm = centimeters, kg = kilograms, BIA = Bioelectric Impedance Analysis,

\* = Group Mean

**Performance assessment**. Exercise performance was evaluated as the time taken to complete the abbreviated OC. The normal OC consists of 21 elements and is usually completed in 7 to 8 min. Our purpose was to assess the effects of Cr supplementation on performing a short-term anaerobic task in a field setting so the OC was reduced to four elements that required about 2 min to complete. The elements consisted of (1) scaling a

3-m high wooden wall, (2) scaling and descending a 10-m high cargo-net rope ladder, (3) climbing a 5-m high rope and transferring to a second rope prior to descent, and (4) an all-out, 100-m sprint to the finish. The distance between obstacles was about 40 meters.

Experimental protocol. Prior to group assignment, each study participant completed two-familiarization runs of the OC separated by 20 min of rest. Each study participant was encouraged to complete the trial run as fast as possible. Only one individual was allowed to run the course at a time to reduce between-subject competition and eliminate interference during negotiation of the OC elements. On a separate day following the two familiarization trial runs, the subjects performed a baseline OC run (control) that was used for comparison with the criterion time-trial run (post-treatment). Prior to the control trial and post-treatment OC trial, each individual was allowed a 10 to 15 min warm-up of their choice.

Prior to group assignment, all subjects were rank-ordered based on their best familiarization trial run and then alternately placed into an A or B group. After completion of the control trial, each participant received 20 vials containing either Cr monohydrate or a polycose placebo (PL), in a double-blind fashion, based on a preassigned number. A technician, not involved with the study, pre-assigned the vials based on group assignment only. The code for group assignment was stored at the Defence and Civil Institute for Environmental Medicine in Toronto, Canada, and was not broken until the study was completed. For the Cr group, each vial contained 5 g of artificially sweetened (NUTRASWEET™) commercially available Cr monohydrate (Ultimate Nutrition; Plainville, CT). The purity of the Cr was verified by one of the investigators (II) and found to be chemically pure in comparison with Cr chemical standards. The PL group received vials containing 5 g of polycose. All substances were made similar in taste, texture, color, and volume. Each volunteer was instructed to self-administer 4 of the 5 g vials at regular intervals throughout the day by dissolving the vial contents into 2 cups of a warm, non-caffeinated drink of their choice. The study participants were asked to follow this regimen for 5 days until all of the vials were consumed. This dose of Cr

(20 g Cr/d for 5 days) has been shown to result in significant increases in intramuscular Cr stores (23).

The day after consuming the final dose of Cr or PL, subjects were asked to report to the OC for the post-treatment OC trial. All volunteers were asked to refrain from alcohol consumption and hard exercise for at least 24 hr before both the control and post-treatment OC trials. To standardize dietary status, the SEALs were also asked to avoid all food consumption (except water) for 3 hr prior to both the control and post-treatment OC trials. All individuals performed the control and post-treatment OC trials at approximately the same time of day.

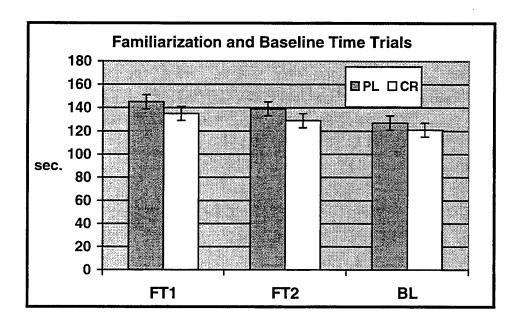
At the OC site, and just prior to the OC trials, each subject had a bioelectrical impedance analysis measurement to estimate body composition (percent body fat) and total body water content. At this time, a urine sample was collected to determine urine specific gravity using a refractometer. At the conclusion of the control and post-treatment OC trials, two 20 µl finger stick blood samples were collected. One sample was collected at 5 min and the other at 10-min post completion of the OC trials. All blood samples were placed immediately into a microcap containing 200 µL of 10% perchloric acid for subsequent blood lactate analysis. The deproteinized extract was assayed using a fluorimetric method (31). The time taken for an individual to complete the OC was quantified using a hand-held stopwatch, accurate to within one-tenth of a second.

Statistical analysis. Initial comparisons, after random assignment into groups, utilized the two sample independent t-test. Group comparisons after the treatment period used a two-factor, repeated measures analysis of variance with one grouping factor (Cr or PL) and one within-subjects factor (control and post-treatment trials). When a significant interaction was observed, Tukey's post hoc comparison procedure for simple effects was used. Statistical significance for this study was set at an alpha level of less than 0.10.

#### **Results**

The Cr and PL groups were very similar for fitness variables, where no significant differences were observed between fat free mass, VO<sub>2peak</sub>, and peak heart rate. After random assignment however, the PL group was significantly older and had greater percent body fat.

Even though all SEALs had trained extensively on the OC prior to the study, the time taken to complete the familiarization OC trials (FT1, FT2) significantly improved (p < 0.03) within each group (Figure 1). The difference in OC run times between groups, however, was not statistically significant nor was there any significant interaction. These results show that a substantial degree of learning occurred before the control (baseline) and post-treatment OC trials, but the learning effect was the same between the groups.



The results of the control and post-treatment OC trials are presented in Table 2. The level of effort exerted between the groups to complete the control and post-treatment OC trials did not differ significantly; as suggested by the blood lactate levels and the OC run times. The lack of any significant difference between the groups' OC run times also supports the contention that the fitness levels and familiarity with the OC was similar

between the groups, even though there were significant differences in percent body fat and age after group assignment.

Table 2. Control (Baseline) versus Post-Treatment Comparisons											
Variable	Creatine (n = 12)				Placebo (n = 12)			ANOVA Results			
	Control	SEM	Post-Tr	SEM	Control	SEM	Post-Tr	SEM	Within	Between	Inter.
Body Wt. (kg)	*78.2	2.2	78.9	2.4	78.9	1.6	78.6	1.6	ns	ns	ns
FFM	68.4	2.7	67.6	3.3	64.1	1.4	64.3	1.2	ns	ns	ns
% Body Fat	12.8	1.9	16.5	1.6	18.6	1.5	18.0	1.5	ns	0.08	ns
TBW (L)	49.8	2.0	48.0	1.7	46.5	1.0	46.7	0.9	ns	ns	ns
ECW (L)	22.8	0.8	22.6	0.7	22.8	0.5	22.8	0.6	ns	ns	ns
ICW (L)	27.0	1.6	25.3	1.1	23.7	0.7	23.8	0.7	ns	0.05	ns
Urine sp.gr.	1.022	0.002	1.026	0.002	1.016	0.002	1.014	0.002	ns	0.03	ns
LA 5' (mmol)	11.5	0.5	11.5	0.5	11.0	0.8	11.5	0.7	ns	ns	ns
LA 10' (mmol)	10.8	0.6	11.2	0.6	11.3	0.8	11.5	0.7	ns	ns	ns
OC run time (s)	120.9	5.0	115.4	4.7	126.8	7.1	122.8	6.1	ns	ns	ns

Abbreviations: ANOVA = Repeated Measures Analysis of Variance, SEM = Standard Error of the Mean, Post-Tr = measured value after 5 days of supplementation with creatine or polycose placebo, Inter. = interaction, FFM = Fat Free Mass as determined by BIA, % Body Fat = % Body Fat as determined by BIA, TBW = Total Body Water, ECW = Extracellular Water, ICW = Intracellular Water, sp.gr. = Specific Gravity, LA 5' = Blood Lactate Levels 5 min after Obstacle Course Run, LA 10' = Blood Lactate Levels 10 min after Obstacle Course Run, mmol = millimoles/liter, OC = Abbreviated Obstacle Course Run Time in Seconds

No significant within-group differences were observed between the control and post-treatment OC time trials. Significant group differences were seen in urine specific gravity and intracellular water as determined by BIA. These differences, however, were related to differences that existed after group assignment and not Cr supplementation. This result becomes evident because there was no significant interaction for any of the measured variables, including the time taken to complete the OC. The lack of significant interaction among observed variables implies no significant treatment effects in the CR supplemented group.

<sup>\* =</sup> Group Mean Value.

#### Discussion

The key result from this study was that 5 days of Cr supplementation at 20 g/d failed to improve OC run times when compared with a PL group with a similar level of training and fitness. This finding is not unlike other studies in the literature (34,36,42), including studies conducted by the U.S. Army and the Royal English Navy that also failed to demonstrate a significant improvement in OC run times (1,47).

For this investigation, we chose a military task that contained elements in which significant improvement had been demonstrated by others using jumping (7), repeated cycling (9) and resistance exercise (45). These studies, on the other hand, were conducted under laboratory conditions using more homogenous test subjects where, presumably, a greater degree of control could be exerted over extraneous variables. Our test subjects tended to be more heterogeneous with respect to percent body fat and age than subjects used in other studies.

Even though we were unable to demonstrate a significant improvement in our performance measure, the Cr group did improve OC run time by 5.5 s (4.5%). The 4.5% improvement seen in the Cr supplemented group is in the range reported by other investigators as statistically significant (22,45). Under those circumstances, the observed variability in the performance measure was lower, implying that smaller mean differences were needed to detect significant differences. In this study, the effect size was about 0.20, which is considered small (11). In order for an effect size of 0.20 to be statistically significant, a sample size of well over 100 individuals would have been required.

There were no significant changes in body weight, fat free mass, or total-body water distribution. Many studies have reported significant changes in body weight of 0.6 kg to 1.1 kg and fat free mass (14,43), while others have not (22). The discrepancy among studies, including our own, may be related to several factors; increased heterogeneity between groups, lack of control over dietary factors, and the inherent variability of the body composition methods employed. The increase in mass seen in some studies has been hypothesized to be due to an increase in water retention, presumably intramuscular

water because of the increased *in situ* concentration of intramuscular Cr (26). Our results do not lend support for this hypothesis, as measured by BIA.

The ability of an individual to increase intramuscular Cr content is dependent on the preexisting baseline levels. Individuals with greater baseline levels of intramuscular Cr prior to Cr supplementation accrue endogenous Cr to a much lesser extent than those with lower intramuscular CR levels (23). It is possible that our subjects had higher baseline intramuscular Cr levels, thus the increase in muscle Cr would be less. This explanation is further supported by the observation that physical training seems to augment the amount of Cr transported into SKM (32,33). Since US Navy SEALs are required to maintain very high levels of fitness (see Table 1), their muscle Cr levels may have already been elevated thus reducing the effectiveness of supplementation. Additionally, the amount of Cr transported into SKM during Cr supplementation has been related to an individual's ability to improve exercise performance (20,21). The failure of the Cr supplemented group to improve performance in the post-treatment OC trial may simply have been a measure of this group's inability to increase intramuscular Cr levels. Also, the 30-day washout period requested among the study participants prior to the beginning of the study, might not have been sufficient to re-establish basal levels of intramuscular Cr (28,41).

In our study, the blood lactate levels were not different between the groups, indicating anaerobic conditions were similar and (at least indirectly) the level of effort was about the same for the control and post-treatment OC trials. The degree of effort and motivation is always a difficult factor to control in studies that require maximum effort. However, our measured blood lactate levels were similar to others in which subjects performed maximal exercise for 2 min (2), indicating that the participants' effort likely was not an issue in this study.

Another aspect of the current study that could have contributed to our lack of significant findings is the length of the supplementation period. Our protocol supplemented subjects with 20 g of Cr for 5 days. This dosage has been shown by some to be sufficient to

significantly increase intramuscular Cr levels (23). But others (26,35) have suggested that supplementary Cr dosages of 20 to 25 g/d for 6 days followed by 30 days of 2 to 5 g/d are needed to achieve and maintain maximal intramuscular Cr concentration. Whether these longer dosage regimens will elicit any further enhancement in performance, however, is largely unknown.

The results of the current investigation indicate that 5 days of dietary supplementation with Cr at 20 g/d had no significant effect on the performance of a military task conducted in an operational setting. Even though there is a physiological basis for Cr to have an ergogenic effect, studies demonstrating a significant improvement in exercise performance conducted in a laboratory setting are not necessarily carried over to exercise performance under field conditions. Further work is needed; however, to determine which military applications would benefit from Cr supplementation. More work is also needed to assess the long-term (> 30 days) effects of high dose Cr supplementation on protein synthesis in humans, protein and nitrogen balance, and the potential for Cr to impact renal function negatively. Until the issue of long-term high dose effects of Cr supplementation are resolved, individuals choosing to use Cr as a dietary supplement should restrict their intake to 20 g/d for 5 days, followed by a maintenance dose of 2 g/d.

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